A recent finding of Armillaria root disease on black oak (*Quercus velutina*) caused by *Armillaria solidipes* in North Carolina, USA

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Introduction

Root disease caused by *Armillaria* spp. is among the leading causes of mortality and lost productivity in forests around the world (Kim et al. 2022). In the northern and western USA, *A. solidipes* (previously known as North American *A. ostoyae*) is considered the most damaging Armillaria root disease pathogen in coniferous forests (Lockman and Kearns 2016). In many regions, climate change is expected to result in an increasing likelihood of damage from Armillaria root disease as trees become more stressed from maladaptation to climate and extreme weather events (Murray and Leslie 2021; Kim et al. 2021). Although *A. solidipes* has been reported in Massachusetts (Brazee and Wick 2009), it had not been previously identified in the southeastern USA until recently (Kim et al. 2023). The objective of this study was to determine distribution of *Armillaria* species in a mixed-hardwood/conifer stand of western North Carolina.

Methods

On a clear and calm day in August 2021, a black oak (*Quercus velutina*), ca. 29 m tall and 64 cm DBH, failed structurally within the Pisgah National Forest near Brevard, North Carolina (Figure 1A). The fallen tree displayed no readily observable, above-ground indications of injury or signs/symptoms of insect- or disease-related damage. Close inspection of the exposed root plate revealed advanced decay inside the roots (Figure 1B) and abundant rhizomorphs attached to the outer root surfaces (Figure 1C). At the point of failure, white mycelial fans were found under the bark of a large primary root (23 cm in diameter) that also showed extensive decay within the root itself (Figure 1D; Kim et al. 2023).

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Figure 1: (A) Failed black oak (*Quercus velutina*) tree, (B) with exposed root plate, (C) covered in abundant black rhizomorphs, and (D) exposed decay column with white mycelial fan.

Rhizomorph samples were collected from the fallen tree and established in culture. Two *Armillaria* isolates were identified (PNF#001R-1 and PNF#001R-2), but somatic pairing tests revealed that these isolates belonged to the same genet (vegetative clone). DNA extracted from fungal mycelia was subjected to PCR and DNA sequencing of the translation elongation factor 1α (*tef1*) gene. Somatic pairing tests were conducted as a second method of species identification. Nine, replicated pairings were conducted using the *Armillaria* isolate (PNF#001R-1) paired with three standard isolates of five other *Armillaria* spp. that occur in North America [*A. solidipes, A. mellea, A. gallica, A. mexicana,* and *Desarmillaria caespitosa* (= *A. tabescens*)] (Kim et al. 2023).

Subsequent surveys were conducted through September 2022 to collect additional samples of *Armillaria* fruiting bodies (basidiomata), rhizomorphs, and mycelial fans from the adjacent mixed-hardwood/conifer forest in the Pisgah National Forest (Figure 2). Samples were collected from diverse tree and shrub species that were symptomatic and asymptomatic for Armillaria root disease, and isolates were established in culture. . *Armillaria* species identification was confirmed via DNA sequencing of *tef1* gene.

Figure 2: (A) Surveys of the area were conducted to collect (B) fruiting body, (C) rhizomorph, and (D) mycelial fan samples of *Armillaria* spp.

Results

Based on *tef1* sequences, both isolates (PNF#001R-1 and PNF#001R-2) were identified as *A. solidipes* (GenBank accession no. OP823701), showing 98% similarity with *A. solidipes tef1* sequences (e.g., MH879015) in GenBank. Somatic pairing tests with *Armillaria* isolate (PNF#001R-1) showed 67% compatibility with *A. solidipes*, but only 0-22% compatibility with the other *Armillaria* species. On the basis of *tef1* and somatic pairing tests, the original *Armillaria* isolate (PNF#001R-1) was identified as *A. solidipes* (Kim et al. 2023).

From a total of 35 additional sample locations (e.g., PNF#002–PNF#036) in the area adjacent to the original *Armillaria* collection (PNF#001), 56 suspected *Armillaria* samples were collected. A majority of sample isolates were established in culture, and potential *Armillaria* isolates were identified based on *tef1* sequences. In total, 45

Armillaria spp. isolates were identified, with 24 identified as *A. mellea*, 16 identified as *A. gallica*, and 5 identified as *A. solidipes* (PNF#003, PNF#033–PNF#36). Somatic pairing tests of all *A. solidipes* isolates, including the original isolate (genet, PNF#001R-1) associated with first detection, showed that these isolates of *A. solidipes* belong to four distinct genets (PNF#001, PNF#003, PNF#033=PNF#034=PNF#035, and PNF#036) (Table 1; Figure 3).

Table 1: Sample isolates identified as *Armillaria solidipes* based on translation elongation factor 1α sequences, and their assignment to genets based on somatic pairing tests.

Armillaria Isolate ID	Sample Date	Sample Type	Host	Genet
PNF#001	8/14/2021	Rhizomorph	*Quercus velutina* (Black Oak)	1
PNF#003	9/7/2021	Rhizomorph	*Fagus grandifolia* (American Beech)	2
PNF#033	9/21/2022	Mycelial Fan	*Kalmia latifolia* (Mountain Laurel)	3
PNF#034	9/22/2022	Rhizomorph	*Quercus montana* (Chestnut Oak)	3
PNF#035	9/23/2022	Rhizomorph	*Oxydendrum arboretum* (Sourwood)	3
PNF#036	9/24/2022	Rhizomorph	*Quercus rubra* (Northern Red Oak)	4

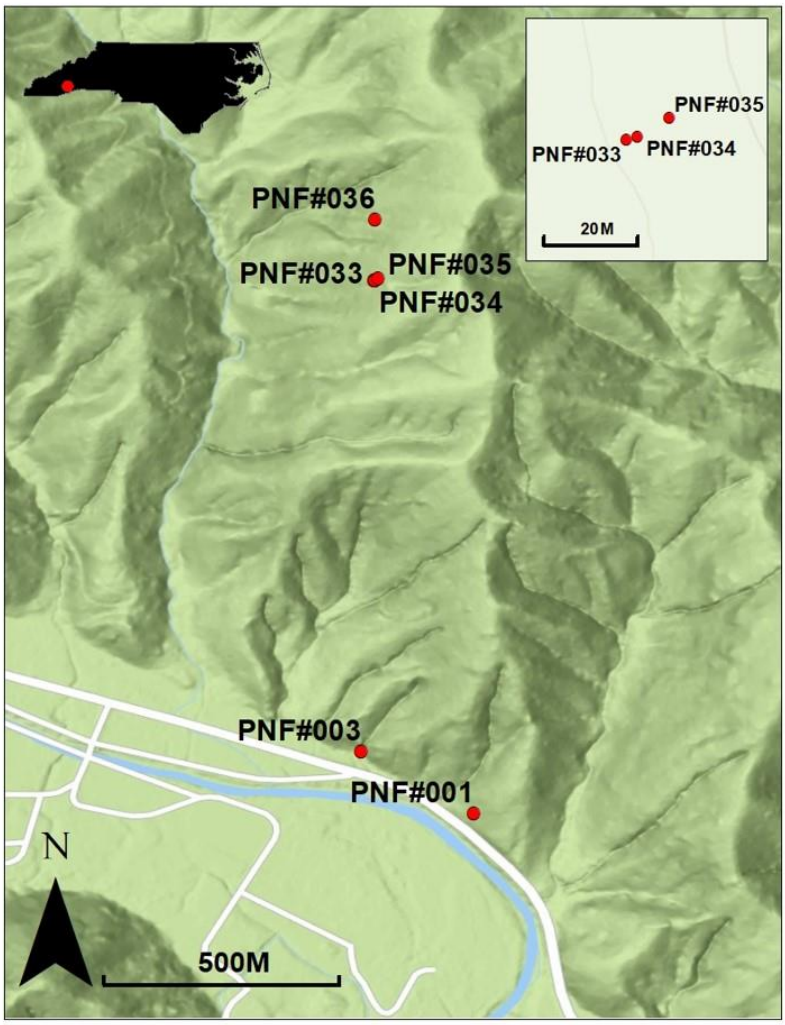

Figure 3: Map showing the location of *Armillaria solidipes* samples collected from Pisgah National Forest in North Carolina, USA.

Conclusions

Based on available knowledge, the original report (Kim et al. 2023) is the first known, confirmed report of *A. solidipes* in North Carolina, which is also the most southerly confirmed presence of *A. solidipes* in eastern North America. Furthermore, our preliminary results indicate that *A. gallica, A. mellea*, and *A. solidipes* can co-occur within a mixed hardwood/conifer stand in the southeastern USA. In western North America, the distribution of *Armillaria* spp. and their ecological behaviors are likely to shift as the climate continues to change (Kim et al. 2021). While it is undetermined if this occurrence of *A. solidipes* is related to a changing climate, continued surveys/studies will help to better understand the range, ecological behaviors, and impact of this Armillaria root disease pathogen in mixed forests across the southeastern USA and beyond.

Acknowledgments

This work was partially funded by USDA Forest Service (FS), State, Private and Tribal Forestry, Forest Health Protection Region 8, USDA FS R&D Pacific Northwest Research Station, and Rocky Mountain Research Station. The findings and conclusions in this publication are those of the authors and should not be construed to represent any official USDA or U.S. Government determination or policy.

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Proceedings of the 68th Western International Forest Disease Work Conference

DoubleTree Hotel

Rohnert Park, California

June 5-9, 2023

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Papers are formatted and have minor editing for language and style but otherwise are printed as they were submitted. The authors are responsible for content.

Citation:

Brooks, R.K. (comps.) 2024. Proceedings of the 68th Western International Forest Disease Work Conference, Rohnert Park, California, 5-9 June 2023. https://www.wifdwc.org/

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